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SUBJECT Best 2000 – Washer Module –
Washer Calibration Software



Attached to this letter you will find a technical bulletin regarding the Washer Module.

The Best 2000 manufacturer, Dynex Technologies, has developed the New Washer Calibration Software (v.2.1) and a new firmware for this module (v.1.14).

With this new software and firmware versions we will be able to calibrate the wash head offset (to compensate for skew of the plate carrier rods) as well as adjust and verify the residual volume in the microplates.

The part number you will require is:

3410-0634	Washer Module Aligment Fixture	WMFIX010
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We extremely recommend reading carefully the instructions and restart the PC after software installation and before using it.

Contact our Technical Service Department to obtain the required software.

Please, do not hesitate to contact us if you need further details.

Tools Required:

- Washer Module Alignment Fixture, WMFIX010
- Flash Software Program
- Calibrated gravimetric balance with 0.01g resolution (minimum range: 0.01 – 100.00g)
- Supply of deionized water or suitable wash buffer
- Supply of clean, flat bottom Micro plates for use in the Verification tests
- A purge tray for bottle calibration
- Red dye solution if you want to verify the residual volume. (The concentration of the read dye solution should measure approximately 2.0 OD at 405 nm measured using 200µl of solution in a micro plate.)

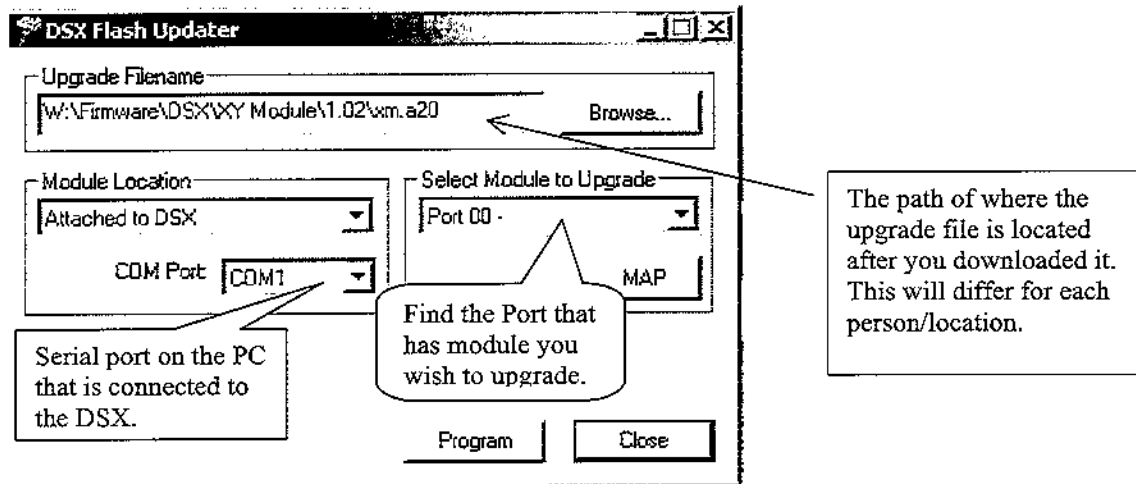
Parts Required:

- Washer firmware file (Washer_1v14.a20)

Washer Module Firmware v.1.14

Instructions on how to upgrade the Washer Module.

1. Open the DSX Flash Program by double clicking on the “DSXFlashSetup.exe” file.
2. Execute the program. (Start-Programs-DSX Flash).
3. You now are in the ‘DSX Flash Updater’ Window.
4. Click the BROWSE button and choose the upgrade file (washer_1v14.a20)
5. Next go to the "Select Module to Upgrade" area and click on MAP.
6. Using the drop down button choose the module (W0 for the washer).
7. Click on the Program button.
8. After the programming is complete, turn the DSX off and back on again and connect with Revelation.



Washer Calibration Software v.2.1

Software Installation Instructions

Unzip program.
Double click on the “setup.exe” icon.
Follow the directions during installation.

New/Changed Features

Added (in conjunction with Washer Module firmware version 1.14) a parallelism correction offset between the plate holder/tray and the washer head (X axis). Also added the ability to verify residual volume and precision.

Introduction

The program allows users to calibrate and verify the operation of Dynex DSX washer bottles along with calibrating and verifying the head offsets as well as verifying residual volume and precision. The program generates a report of all data, calculated results and test results.

Set-up

Ensure that the DSX is turned on and connected to the computer. Ensure that DSX Revelation software is not running, so that communication between this calibration program and the DSX may be established.

When one of the program buttons is clicked and the program cannot establish a communication link to the DSX, an error message reminding the user to power and connect the DSX will be displayed.

Entering User Information

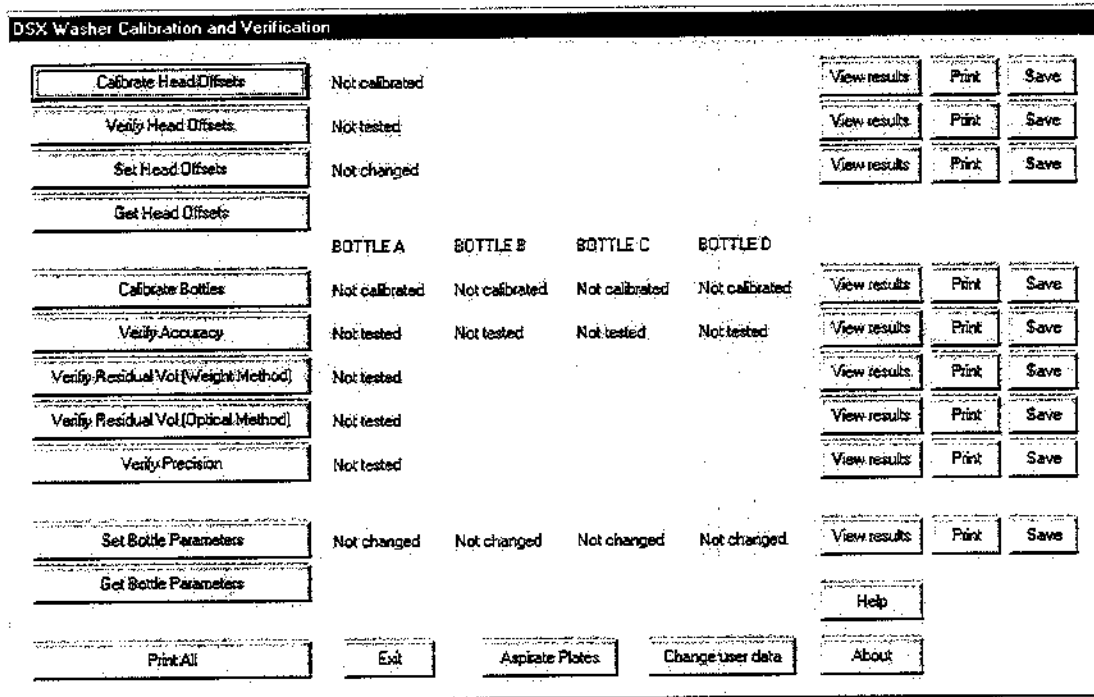
The opening screen will look like this:

Fill in all fields before continuing. Enter the room temperature in °C. This will be used to calculate water density. The program automatically calculates water density based on the specified room temperature. If a calibration fluid other than water is used, click the **Custom** button, enter the fluid name and fluid density in g/ml. When all the data has been entered, select the comm port that is used and click **OK** to continue.

The operator will be prompted to open the DSX cover, fill bottles with calibration fluid and connect all bottles correctly. This step entails connecting each bottle's electrical fittings to the front panel, connecting the tubing Quick Connect fitting to the top of the bottle and threading the dispense tubing through the large dispense valve on the front panel.

Main Program Operation

The main program screen looks like this:



From this screen, the operator may do the following, calibrate head offsets, set or get offsets, calibrate and verify bottles, set or get bottle parameters, edit user data, view and print test reports or exit the program. An **About** button provides information about the program version.

Calibrate Head Offsets

To calibrate the wash head, click on **Calibrate Head Offsets**. Follow the program prompts. At the end of the calibration procedure, new calibration values will be calculated. If these values are numerically valid, the operator will be given the choice to save this new values to memory, or to exit the calibration. If the values are numerically invalid, the program will report that the calibration must be repeated.

After calibration, all results will be logged and may be viewed by clicking the **View Results** button to the right of **Calibrate Head Offset**.

DYNEX Technologies strongly recommends following every parameter change or calibration change with a Verification test.

What happens during a head offsets calibration? The program instructs the operator to insert a calibration fixture. Then the program moves the head to different locations on the calibration fixture. At these location the operator adjusts the head using the program so that the pins in the head are lined up to the fixture. These calibration points are used to generate four calibration values. The calibration values include the bottle X offset, Y offset, Z1 offset, Z12 offset.

Numerically valid values for these parameters are:

$0 < X \text{ offset} \leq 100$

$0 < Y \text{ offset} \leq 100$

$0 < Z1 \text{ offset} \leq 100$

$0 < Z12 \text{ offset} \leq 100$

When the program is started and before any calibration activities have occurred, the words "Not Calibrated" will appear below each bottle letter. After calibration, this text will change to "Complete" or "Incomplete", depending on the outcome of the calibration.

Verify Head Offsets

Use this option to verify the head offsets. The program will run a wash aspiration cycle using the calibration fixture as a plate. The user will observe if the pins touch the calibration fixture. If they do the test Fails if the don't the test Passes

Set Head Offsets

Use this option to manually set new head offsets. The program will report the current settings for the head, as well as the factory default settings, and allows the operator to input new values.

WARNING! Setting new head offset will alter the performance of the washer. Using values without performing a calibration and subsequent verification is not recommended and may cause invalid results.

Get Head Offsets

Use this option to view the head offsets. The program will report the current head offsets, as well as the factory default settings. Dynex starts every calibration with these default settings. These defaults may or may not be the settings your DSX washer module left the factory with; they are the starting point for every calibration and are provided for informational purposes only.

Calibrate Bottles

To calibrate a bottle, click on **Calibrate bottles**. Enter the bottle letter of the bottle to be calibrated and then follow the program prompts. At the end of the calibration procedure, new calibration values will be calculated. If these values are numerically valid, the operator will be given the choice to save these new values to memory, or to exit the calibration. If the values are numerically invalid, the program will report that the calibration must be repeated.

After calibration, all results will be logged and may be viewed clicking the **View Results** button to the right of **Calibrate bottles**.

DYNEX Technologies strongly recommends following every parameter change or calibration change with a Verification test.

Note: When a bottle is calibrated in one particular position on the DSX, the calibration values correspond to *that physical location*. For example, if a bottle is calibrated in position B and then

moved to position C, the calibration does not automatically move with the bottle. **DYNEX Technologies strongly recommends that a bottle be recalibrated and verified if its physical location on the DSX is changed.**

What happens during a bottle calibration? The DSX will dispense fluid into a reservoir and the operator weighs the fluid. The program converts weight to volume. Two calibration points are used to generate a linear equation that describes the calibration of a bottle. The calibration values for a bottle include the bottle speed, and the slope and intercept of the line describing the calibration.

Dynex recommends using a low-sided, Micro plate-sized vessel for the calibration because a typical Microtiter plate cannot hold the volume dispensed in the calibration. If the operator does not have access to an appropriate fluid reservoir, it is possible to carefully remove the dispense head from the washer arm and hold the head over an alternative weighing vessel. Simply ensure that the dispense and aspirate tubing are not kinked, and that the dispense tubing remains threaded through the small dispense pinch valve on the front edge of the washer module.

The DSX washer module will move the dispense head over to the middle of the fluid reservoir, dispense, move slightly left and then return to dispense more fluid. All in all, five fluid dispenses occur for each of the two calibration points in order to minimize dispense-to-dispense errors.

After all calibration operations are completed, the program will report the new calculated slope and intercept values for the bottle being tested. Numerically valid values for these parameters are:
 $0 < \text{bottle speed} \leq 250$ (DYNEX Technologies specifies a standard bottle speed of 190)
 $0 < \text{slope} \leq 6.5$ (typically this value will be in the range of 1.5 to 4.5)
 $0 < \text{intercept} \leq 512$ (typically this value will be in the range of 20 to 60)

The above ranges are provided for informational purposes only. **WARNING! Using these values without performing a calibration and subsequent verification is not recommended and may cause invalid results.**

Note: This calibration program automatically multiplies the calculated slope value by a factor of twenty. This is merely a way to increase the sensitivity of the parameter, while saving it as an integer value in the DSX washer module memory. The calculated value is always *reported* as a decimal value, but it is always *stored* as an integer, hence the "x20" factor. The printed calibration report notes this calculation as well.

When the program is started and before any calibration activities have occurred, the words "Not Calibrated" will appear below each bottle letter. After calibration, this text will change to "Complete" or "Incomplete", depending on the outcome of the calibration.

Verify Accuracy

To verify a bottle, click on **Verify Accuracy**. Enter the bottle letter of the bottle to be verified and then follow the program prompts. At the end of the verification procedure, a "Pass" or "Fail" result will be reported. All test results will be logged and may be viewed clicking the **View Results** button to the right of **Verify Accuracy**.

What happens during a bottle verification? The DSX washer module will dispense 300µl/well across a 96 well plate. This fluid is weighed by the operator, and the weight is converted into a

mean well volume. The difference between the actual delivered well volume and the target of 300µl is calculated.

A passing result is %Error <= 10%. This means that for the program to report a PASS result, the actual average dispensed well volume must fall within the range of 270 – 330µl/well.

When the program is started and before any verification activities have occurred, the words "Not Tested" will appear below each bottle letter. After verification, this text will change to "Pass" or "Fail", depending on the outcome of the verification.

Verifv Residual Volume (Weight Method)

To verify the washers whole plate aspiration performance, click on **Verify Residual Volume (Weight Method)**, then follow the program prompts. At the end of the verification procedure, a "Pass" or "Fail" result will be reported. All test results will be logged and may be viewed clicking the **View Results** button to the right of **Verification Residual Volume (Weight Method)**.

What happens during a residual volume (weight method) verification? An empty plate is weight by the operator. This plate is inserted in the washer and then the washer will dispense 300µl/well across a 96 well plate. This fluid is aspirated the plate is removed and weighed again. The weight is converted into a mean residual volume per well. This test is done for both a super sweep and a normal sweep aspiration.

A passing result is residual volume <= 3uL for super sweep and <= 5uL for normal sweep.

When The program is started and before any verification activities have occurred, the words "Not Tested" will appear next to **Verify Residual Volume (Weight Method)**. After verification, this text will change to "Pass" or "Fail", depending on the outcome of the verification.

Verifv Residual Volume (Optical Method)

To verify the washers row, column, and whole plate aspiration performance, click on **Verify Residual Volume (Optical Method)**, then follow the program prompts. At the end of the verification procedure, a "Pass" or "Fail" result will be reported. All test results will be logged and may be viewed clicking the **View Results** button to the right of **Verification Residual Volume (Optical Method)**.

What happens during a residual volume (optical method) verification? An empty plate is weighed by the operator. This plate is filled with red dye solution using a pipette. Then this plate is inserted in to the washer. The fluid is then aspirated. The plate is weighed again and put into the washer. Then the washer will dispense 200µl/well across a 96 well plate. This plate is then transferred to the reader where it is read. The weight and optical measurements are converted into a mean residual volume per well. This test is done for both a super sweep aspiration and a normal sweep aspiration.

A passing result is residual volume <= 3uL for super sweep and <= 5uL for normal sweep.

When the program is started and before any verification activities have occurred, the words "Not Tested" will appear next to **Verify Residual Volume (Optical Method)**. After verification, this text will change to "Pass" or "Fail", depending on the outcome of the verification.

Verifv Precision

To verify the precision of the washer, click on **Verify Precision**, then follow the program prompts. At the end of the verification procedure, a "Pass" or "Fail" result will be reported. All test results will be logged and may be viewed by clicking the **View Results** button to the right of **Verify Precision**.

What happens during precision verification? The washer bottle is filled with red dye solution. The DSX washer module will dispense 250µl/well across a 96 well plate. This plate is then transferred to the reader where it is read. The CV of the plate measurement is calculated.

A passing result is $\leq 5\%$ CV.

When the program is started and before any verification activities have occurred, the words "Not Tested" will appear next to **Verify Precision**. After verification, this text will change to "Pass" or "Fail", depending on the outcome of the verification.

Get bottle Parameters

Use this option to view the calibration parameters for a particular bottle. These parameters include bottle speed, slope and intercept. The program will report the current settings for a bottle, as well as the factory default settings. Dynex starts every calibration with these default settings. These defaults may or may not be the settings your DSX washer module left the factory with; they are the starting point for every calibration and are provided for informational purposes only.

Set bottle Parameters

Use this option to manually set new calibration parameters for a particular bottle. These parameters include bottle speed, slope and intercept. The program will report the current settings for a bottle, as well as the factory default settings, and allows the operator to input new values.

WARNING! Setting new calibration parameters will alter the performance of the bottle. Using values without performing a calibration and subsequent verification is not recommended and may cause invalid results.

DYNEX Technologies strongly recommends following every parameter change or calibration change with a Verification test.

If calibration parameters have not been manually altered using the **Set bottle Parameters** function, the words "Not Changed" will appear below each bottle letter. If calibration parameters are altered using the **Set bottle Parameters** function, this text will change to "Changed".

Print Report

Use this option to print the results from all calibrations and tests. The operator should sign each report on the line provided in order to maintain documentation needed for Good Laboratory Practices.

Aspirate Plate

Use this option to remove the liquid from the plates that were used for the test.